Docket No.: 146392005600
Client Reference No.: P2026R1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Jun LIU et al.

Application No.: 10/813,483 Confirmation No.: 5594

Filed: March 29, 2004 Art Unit: 1644

For: HIGH CONCENTRATION ANTIBODY AND PROTEIN FORMULATIONS Examiner: Y. Kim

DECLARATION OF JUN LIU PURSUANT TO 37 C.F.R. §1.132

MS RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Jun Liu, declare as follows:

1. I am an inventor of the above-referenced patent application, under the name of Jun Liu.

2. I have a Ph.D. in biochemistry from University of New Hampshire. I am currently a senior scientist at Genentech, Inc., a position I have held since 2005. My scientific expertise includes the fields of drug formulation, delivery and biophysical characterization. A copy of my curriculum vitae is enclosed.

3. I am familiar with the above-referenced patent application. I have also reviewed the final Office Action mailed June 4, 2008, and references cited by the Examiner. In particular, the

Examiner alleges that claims 1, 3-8, 20 and 22-25 in the above-referenced patent application are obvious over WO 97/26909 in view of U.S. Pat. No. 5,994,511 because it would have been obvious to one of the ordinary skill in the art at the time the invention was made to stabilize rhuMAbE25 using a formulation comprising a buffer comprising histidine, arginine, and polysorbate as taught by the WO 97/26909 publication. The Examiner further rejects claims 1, 3-8, 20 and 22-25 on the ground of nonstatutory obviousness-type double patenting over claims 1-4, 7-13, 22-27, 31-34, 37-42, 48, 51-56, 58 and 59 of U.S. Pat. No. 6,875,432 in view of US 2004/109243. I respectfully disagree with Examiner's conclusions.

4. Proteins having different amino acid sequences, secondary and tertiary structures, and functions would have different characteristics and would require different formulations to maintain stability and ensure ease of administration. rhuMAbE25 is an antibody that has many unique characteristics, and a specific combination of excipients and pH condition need to be developed to address the unique problems associated with developing a stable liquid formulation for this protein. The following experiments conducted at Genentech, Inc. under my supervision demonstrate the unique properties of rhuMAbE25 and selection of excipients and conditions for forming stable liquid formulations for rhuMAbE25.

5. As protein concentration increases, rhuMAbE25 liquid formulation forms a turbid and cloudy solution. To identify an excipient that reduces the turbidity of a liquid formulation for rhuMAbE25, various excipients have been tested. As shown in Example 5 of the above-reference patent application, a formulation containing arginine-HCl has the least turbidity. Turbidity of rhuMAbE25 in a liquid formulation was compared to other antibodies, such as an anti-HER2 antibody and an anti-CD11a antibody, in the same liquid formulation. Each antibody was formulated at 117 mg/ml in 30 mM histidine buffer at pH 6.0 with or without 200 mM arginine-HCl. Optical density (OD) measurements of undiluted antibody formulations were taken on a Hewlett-Packard spectrophotometer, with a 1-cm quartz cuvette, using Milli-Q water as a reference. The OD values from 360 nm to 340 nm were obtained, averaged, and reported as an average OD at 350 nm for turbidity value for an antibody formulation. Turbidity values for each antibody formulation with or without 200 mM arginine-HCl are summarized in the table below. As indicated

in the table, in the absence of arginine-HCl, only rhuMAbE25 liquid formulation had a turbidity value over 0.3; and the turbidity value was reduced to 0.2474 by having 200 mM arginine-HCl in the formulation. In contrast, for the formulation containing the anti-HER2 antibody, the turbidity value was increased from 0.21648 to 0.2909 when arginine-HCl was added into the formulation. Data in this table show that high turbidity problem is unique to rhuMAbE25, and arginine-HCl, which reduces turbidity for rhuMAbE25 in a liquid formulation, may not be useful as an excipient for other antibodies or proteins. The data further indicate that different antibody has different characteristics in liquid formulation and a formulation that works for one antibody or a protein may not be useful for another antibody or protein.

	Turbidity value (without arginine-HCl)	Turbidity value (with 200 mM arginine-HCl)
rhuMAbE25	0.3471	0.2474
Anti-HER2 antibody	0.21628	0.2909
Anti-CD11a antibody	0.2165	0.1787

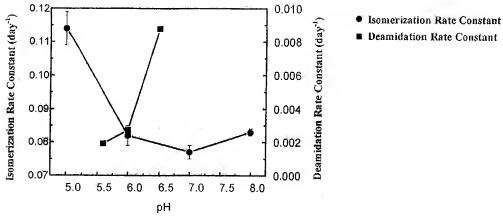
6. To optimize a liquid formation for a therapeutic antibody, it is important to select excipients and conditions in order to maintain antibody stability in the formulation for a longer shelf life. The effect of pH on chemical stability of rhuMAbE25 in a liquid formulation was studied.

7. Isomerization rate constants (for Asp-isoAsp) of rhuMAbE25 at different pH were tested. rhuMAbE25 samples were prepared at 20 mg/ml in formulation buffers at different pH. The formulation buffers contain 150 mM sodium chloride and 20 mM acetate pH 5.0, 20 mM histidine pH 6.0, 20 mM phosphate pH 7.0, or 20 mM phosphate pH 8.0. Isomerization was measured on day 0, day 1, day 2, day 3, day 6, and day 8 after storing the samples at 50°C. Isomerization reaction was determined using a papain digested hydrophobic interaction chromatography (HIC) method. Each sample was diluted to 5 mg/ml using the formulation buffer, then mixed with 10 mM cysteine and 1 M Tris, 40 mM EDTA at pH 7.4 and 0.1 mg/ml papain. The diluted samples were then incubated at 37°C for 2 hours. The digestion was stopped by adding 100 ml of antipain to each tube. Fab fragments containing Asp, IsoAsp, succinimide, free sulfhydryl and disulfide forms were

separated by HIC and the relative amount of Fab fragment in each form was measured. The HIC experiments were conducted using a TSK Phenyl-5PW (7.5 x 75 mm) column and an HP 1100 liquid chromatography system. The column was loaded with ~ 28 mg of papain digested samples and eluted with a concentration gradient of ammonium sulfate in 20 mM Tris buffer from 2 M to 0 M. The peaks were monitored at 214 nm by a UV detector. The data were collected and analyzed using an HP Chemstation software. The isomerization rate constants were obtained by fitting the stability data as a pseudo first order reaction.

8. Deamidation rate constants of rhuMAbE25 at different pH were tested. rhuMAbE25 samples were prepared at 150 mg/ml in 200 mM arginine-HCl, 20 mM histidine-HCl, at pH 5.6, 6.0 or 6.5. Deamidation was measured on day 0, day 14, and day 28 after storing the samples at 30°C. Deamidation reaction was determined using an ion exchange chromatography (IEC) method. The samples were prepared by carboxypeptidase B (CpD) digestion to remove terminal lysine. Each sample was diluted to about 1 mg/ml with 20 mM MES buffer at pH 6.1. CpD was added to samples to make a final concentration of 1% (w/w). The samples were then incubated for 20 minutes at 37°C and stored at 5°C until analyzed. The IEC experiments were carried out using a Dionex ProPac WCX-10 (4 x 250 mm) column and an HP 1100 liquid chromatography system. The column was loaded with ~ 50 µg of CpD digested samples and run at 0.8 ml/min. The protein was eluted with a linear gradient of sodium chloride in 20 mM MES buffer at pH 6.1. The eluate was monitored at 280 nm by a UV detector. The deamidation rate constants were obtained by fitting the stability data as a pseudo first order reaction.

9. As shown in the graph below, the isomerization rate constant of rhuMAbE25 in liquid formulations decreased when the pH was higher than 5.5; but the deamidation rate constant increased significantly when the pH was higher than 6.0. Therefore, the preferred pH range for a stable liquid formulation for rhuMAbE25 is from pH 5.5 to pH 6.0.



10. The data described above demonstrates that turbidity problem is unique to rhuMAbE25, and thus, one skilled in the art would not have been motivated to use a liquid formulation developed for factor IX for rhuMAbE25 and would not reasonably expect that a liquid formulation comprising arginine, histidine, and polysorbate disclosed for factor IX could solve the turbidity problem of rhuMAbE25 in liquid formulations. In addition, the data indicate that, in order to achieve the required stability for rhuMAbE25, the preferred pH for a liquid formulation is from 5.5 to 6.0. This pH range is not the preferred pH range for the formulations (for proteins including rhuMAbE25) claimed in U.S. Pat. No.6,875,432. Thus, one skilled in the art would not have been motivated to choose this pH range for a rhuMAbE25 liquid formulation and would not reasonably expect that this pH range could maintain stability for rhuMAbE25 in liquid formulations as claimed in the above-referenced patent application. Accordingly, I respectfully disagree with Examiner's conclusions stated in the Office Action.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Sep 4, 2008
Date

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EXPERIENCES

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Research Assistant (1986-1989)
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EDUCATION

Ph.D. in Biochemistry *May, 1993*
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Dissertation: Characterization of Biglycan and Decorin Self-association.

B.E. in Biochemistry and Biochemical Engineering *August, 1986*
East China University of Chemical Engineering and Technology, Shanghai, China

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2. Wakankar AA., Liu J., Velde DV., Wang YJ., Shire SJ., Borchardt RT., The Effect of Co-solutes on the Isomerization of Aspartic Acid Residues and Conformational Stability in a Monoclonal Antibody. 96(7):1708-18, 2007 Jul.
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- Liu, J and Shire, S. J. High Concentration Antibody and Protein Formulation, WO Patent (WO2004091658)
- Gwee, S. and Liu, J. High Concentration Liquid Formulation for 2C4, filed in 2004

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- Xolair Liquid, IND Amendment, 2005
- rhuMAb 2C4, IND Amendment, 2004
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- rhuMAb 2C4, IND Amendment, 2002

OUTSIDE PRESENTATION

- Liu J (2007) Strategies for Developing High Concentration Monoclonal Antibody Formulation, Invited Speaker at FIP World Congress of Pharmacy and Pharmaceutical Sciences
- Liu J (2007) Challenges and Issues in the Development and Manufacture of High Concentration Antibody Formulations, Invited Speaker at Keystone antibody conference
- Liu J (2007) Formulation and Analytical Challenges in the Development of High Concentration Monoclonal Antibody, Speaker at AAPS biotech national meeting
- Liu, J (2006) Improving the Chemical Stability of a Theraperutc Monoclonal Antibody for Aspartate Isomerization, Invited Speaker at Protein Engineering for Biotherapeutics, San Diego.
- Liu J (2006) Challenges of Transition from an Approved Lyophilized Formulation to a Liquid Formulation, Invited Speaker at IBC Formulation Strategies for Protein Therapeutics meeting, San Francisco.
- Liu, J. (2005) Impact of Reversible Self-Association of Protein on Viscosity and Nucleation of a Monoclonal Antibody, IBC Formulation Strategies for Protein Therapeutics, Boston.
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PROFESSIONAL SOCIETIES/ACTIVITIES

American Association of Pharmaceutical Scientists
American Chemical Society
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